

Supplemental Data

The single-cell epigenomic and transcriptional landscape of immunity to influenza vaccination

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DataS1 – Subject and vaccine information, related to STAR Methods

Subject information

TIV

Treatment	Participants (no.)	Median Age (range)	Gender (%)	Race (%)
Antibiotics	10	29 (24 - 38)	Male (70%) Female (30%)	White (50%) Black or African American (30%) Other (20%)
Control	11	27 (24-35)	Male (64%) Female (36%)	White (63%) Black or African American (27%) Other (10%)

H5N1/H5N1+AS03

Vaccine	Participants (no.)	Median Age (range)	Gender (%)	Race (%)
H5N1	16	26 (22-40)	Male (44%) Female (56%)	White (44%) Black (25%) Asian (19%) Other (12%)
H5N1+AS03	34	28 (21-44)	Male (38%) Female (62%)	White (77%) Black (18%) Asian (3%) Other (2%)

Vaccine information

TIV

Vaccine	Vaccine Brand/Season	H1N1 strain	H3N2 strain	B strain
TIV	Fluzone 2014-2015	A/California/07/2009	A/Texas/50/2012	B/Massachusetts/02/2012

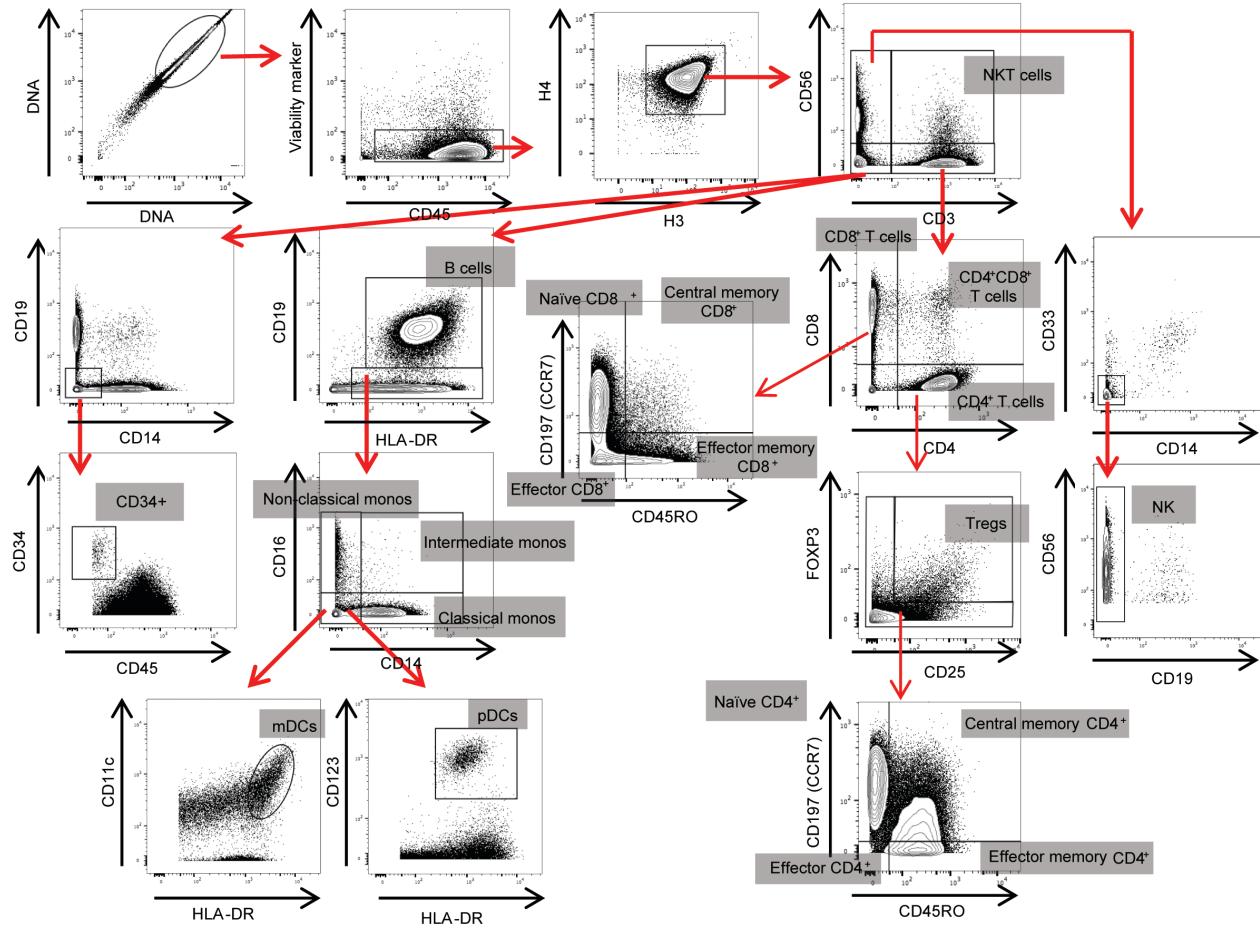
DataS2 – EpiTOF panel and gating, related to STAR Methods

EpiTOF antibody panel

Panel 1					Panel 2				
Metal	Marker	Manufacturer	Type	Clone	Metal	Marker	Manufacturer	Type	Clone
89Y	CD45	Fluidigm	Mouse IgG1	HI30	89Y	CD45	Fluidigm	Mouse IgG1	HI30
141Pr	H3	CST	Rabbit mAb	D1H2	141Pr	H3	CST	Rabbit mAb	D1H2
142Nd	γ-H2AX	CST	Rabbit mAb	20E3	142Nd	Arg-me1	Abcam	Mouse IgG1	5D1
143Nd	H2BK5ac	CST	Rabbit mAb	D5H1S	143Nd	Arg-me2 (sym)	CST	Rabbit mAb	13222
144Nd	H3S10ph	Active Motif	Mouse IgG1	MABI 0312	144Nd	H3K4me2	Active Motif	Mouse IgG1	MABI 0303
145Nd	CD4	BioLegend	Mouse IgG1	RPA-T4	145Nd	CD4	BioLegend	Mouse IgG1	RPA-T4
146Nd	CD8	BioLegend	Mouse IgG1	SK1	146Nd	CD8	BioLegend	Mouse IgG1	SK1
147Sm	H4K5ac	Active Motif	Mouse IgG1	MABI 0405	147Sm	H3K9me2	Biolegend	Mouse IgG1	5E5-G5
148Nd	CD34	BD	Mouse IgG1	8G12	148Nd	CD34	BD	Mouse IgG1	8G12
149Sm	Cleaved H3 (Thr22)	CST	Rabbit mAb	D7J2K	149Sm	H3K9me1	Biolegend	Mouse IgG1	7E7.H12
150Nd	H3.3S31ph	Active Motif	Mouse IgG2b	1A8G10	150Nd	H3K36me3	RevMab	Rabbit mAb	RM155
151Eu	H3K23ac	RevMab	Rabbit mAb	RM169	151Eu	H3K27me1	Active Motif	Mouse IgG2a	MABI 0321
152Sm	H3K9ac	Active Motif	Mouse IgG2a	2G1F9	152Sm	Arg-me2 (asy)	CST	Rabbit mAb	13522
153Eu	H2BS14ph	CST	Rabbit mAb	D67H2	153Eu	H3K36me2	Active Motif	Mouse IgG1	MABI 0332
154Sm	H2AK119ub	CST	Rabbit mAb	D27C4	154Sm	H3K27me3	Active Motif	Mouse IgG1	MABI 0323
155Gd	CD11c	BioLegend	Mouse IgG1	Bu15	155Gd	CD11c	BioLegend	Mouse IgG1	Bu15
156Gd	H3K18ac	RevMAb	Rabbit mAb	RM166	156Gd	H4K20me2	Active Motif	Mouse IgG1	MABI 0422
158Gd	H3K56ac	Active Motif	Mouse IgG1	12.1	158Gd	H3.3	Abcam	Rabbit mAb	EPR17899
159Tb	CD197	Biolegend	Mouse IgG2a	G043H7	159Tb	CD197	Biolegend	Mouse IgG2a	G043H7
160Gd	PADI4	OriGene	IgG2a	OTI4H5	160Gd	H4K20me3	BioLegend	Mouse IgG1	6F8-D9
161Dy	H2BK120ub	CST	Rabbit mAb	D11	161Dy	Macro-H2A	Millipore	Mouse IgG2b	14G7
162Dy	Crotonyl-Lys	PTM Biolabs	Mouse IgG	4D5	162Dy	H3K4me3	Life	Rabbit IgG	G.532.8
163Dy	H3R2cit	Abcam	Rabbit mAb	EPR17703	163Dy	H2A.Z	Abcam	Rabbit mAb	[EPR6171(2)(B)]
164Dy	H3K14ac	CST	Rabbit mAb	D4B9	164Dy	H3K36me1	Abcam	Rabbit mAb	EPR16993
165Ho	FOXP3	BD	Mouse IgG1	259D/C7	165Ho	FOXP3	BD	Mouse IgG1	259D/C7
166Er	CD123	BD	Mouse IgG1	9F5	166Er	CD123	BD	Mouse IgG1	9F5
167Er	CD45RO	BioLegend	Mouse IgG2a	UCHL1	167Er	CD45RO	BioLegend	Mouse IgG2a	UCHL1
168Er	H4K16ac	CST	Rabbit mAb	E2B8W	168Er	H4K20me1	Active Motif	Mouse IgG	5E10-D8
169Tm	CD25	Fluidigm	Mouse IgG1	2A3	169Tm	CD25	Fluidigm	Mouse IgG1	2A3
170Er	CD3	BioLegend	Mouse IgG1	UCHT1	170Er	CD3	BioLegend	Mouse IgG1	UCHT1
171Yb	CD14	Biolegend	Mouse IgG2a	M5E2	171Yb	CD14	Biolegend	Mouse IgG2a	M5E2
172Yb	CD56	BD	Mouse IgG2b	NCAM16.2	172Yb	CD56	BD	Mouse IgG2b	NCAM16.2
173Yb	H4	Abcam	Mouse IgG1	ab31830	173Yb	H4	Abcam	Mouse IgG1	ab31830
174Yb	H3K27ac	Active Motif	Mouse IgG1	MABI 0309	174Yb	CENP-A	MBL	Mouse IgG1	3-19
175Lu	CD19	BioLegend	Mouse IgG1	HIB19	175Lu	CD19	BioLegend	Mouse IgG1	HIB19
176Yb	HLA-DR	BioLegend	Mouse IgG2a	L243	176Yb	HLA-DR	BioLegend	Mouse IgG2a	L243
209Bi	CD16	Fluidigm	Mouse IgG	3G8	209Bi	CD16	Fluidigm	Mouse IgG	3G8

DataS2 – EpiTOF panel and gating, related to STAR Methods (Continued)

EpiTOF gating scheme



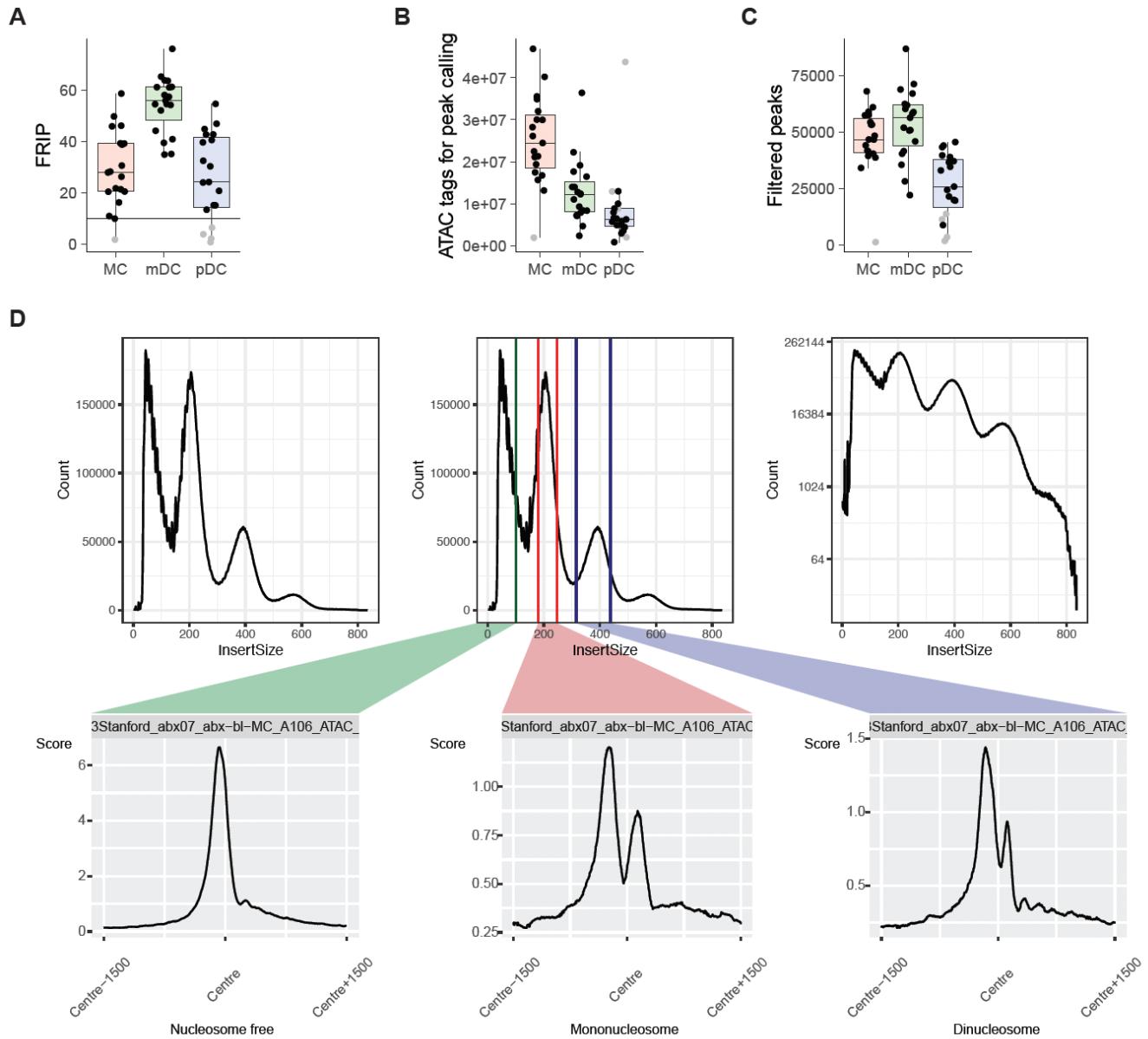
DataS3 - Vaccine-induced epigenomic changes by bulk ATAC-seq and RNA-seq, related to Figure 3

(A) Cell abundance as determined by flow cytometry during FACS sorting. Wilcoxon signed-rank test was used to compare groups.

(B) Abundance of CD16⁺CD14⁺ monocytes within the sorted CD14⁺ monocyte compartment. Wilcoxon signed-rank test was used to compare groups.

(C) Boxplots showing different QC parameters for bulk ATAC-seq samples. Samples in grey were excluded from analysis based on FRIP threshold shown in the left panel.

(D) Fragment size distribution plots and TSS enrichment plots from a representative ATAC-seq sample.



DataS4 - ScATAC-seq and scRNA-seq analysis of immune response to TIV, related to Figure 4

(A) UMAP representation of single immune cells using scATAC-seq after pre-processing. Color indicates clusters with manually annotated cell type identity.

(B) Boxplot showing the number of unique fragments per cell in each cluster.

(C) Heatmap showing chromVAR TF accessibility values per cluster for the top 60 most variable TFs. Accessibility was normalized by row.

(D) Shown is the cell fraction by cluster for each vaccine time point (left) and subject (right) using scATAC-seq data.

(E) UMAP representation of single immune cells using scRNA-seq after pre-processing. Color indicates clusters with manually annotated cell type identity.

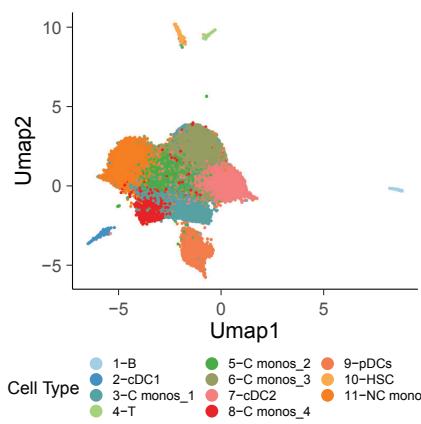
(F) Per-cell QC metrics. After preprocessing, qc metrics were calculated for each cell in each cluster. Shown are the RNA counts (top), unique genes (middle), and mitochondrial RNA fraction (bottom) per cell.

(G) Top7 distinctive genes per cluster. After QC filtering, we calculated the differentially expressed genes (DEGs) in each cluster compared to all other cells. Heatmap shows the expression levels of the top 7 DEGs in up to 500 randomly sampled cells from each cluster.

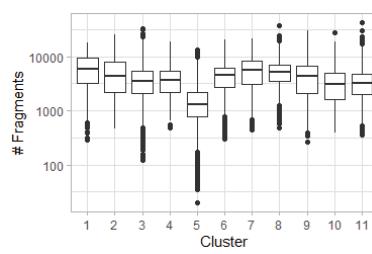
(H) Shown is the cell fraction by cluster for each vaccine time point (left) and subject (right) using scRNA-seq data.

DataS4 - ScATAC-seq and scRNA-seq analysis of immune response to TIV, related to Figure 4 (Continued)

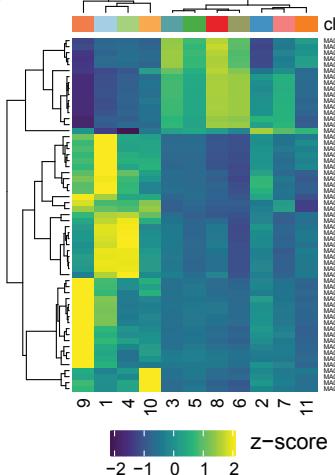
A



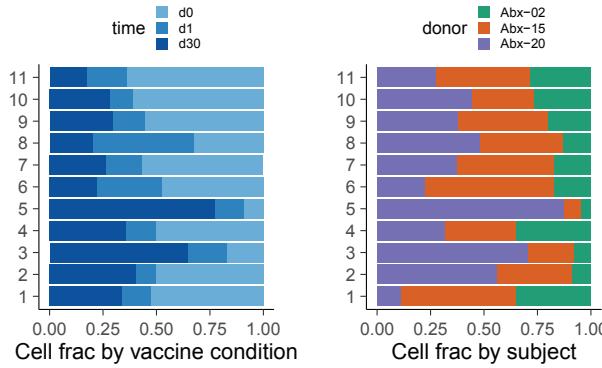
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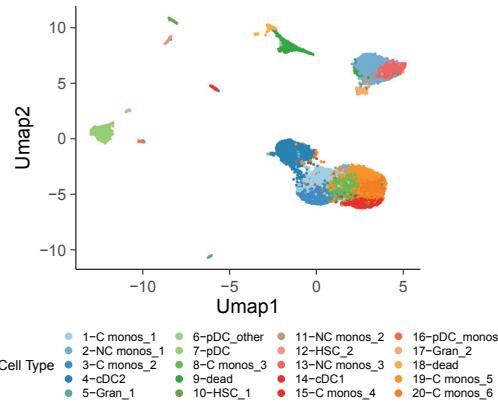
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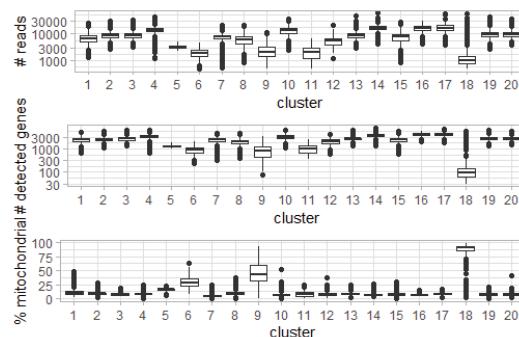
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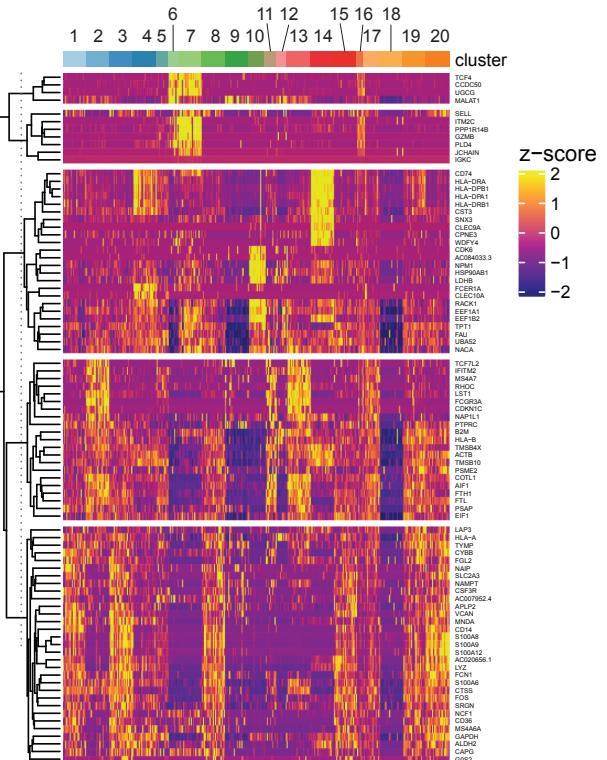
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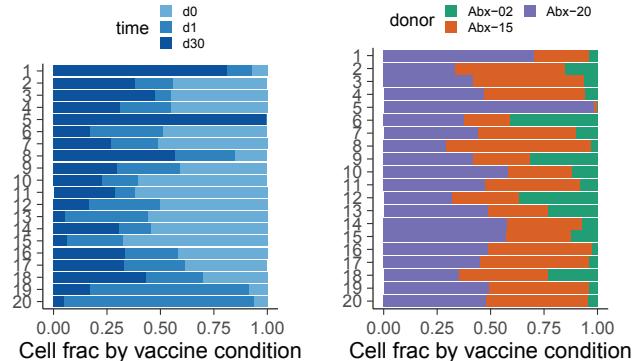
F



G



H



Data S5 - ScATAC-seq and scRNA-seq analysis of immune response to H5N1/H5N1+AS03, related to Figure 5

(A) UMAP representation of single immune cells using scATAC-seq after pre-processing. Color indicates clusters with manually annotated cell type identity.

(B) Boxplot showing the number of unique fragments per cell in each sample before downsampling (top) and after downsampling (middle), as well as per cluster after downsampling (bottom).

(C) Heatmap showing chromVAR TF accessibility values per cluster for the top 60 most variable TFs. Accessibility was normalized by row.

(D) Shown is the cell fraction by cluster for each vaccine condition (left) and subject (right) using scATAC-seq data.

(E) UMAP representation of single immune cells using scRNA-seq after pre-processing. Color indicates clusters with manually annotated cell type identity.

(F) Per-cell QC metrics. After preprocessing, qc metrics were calculated for each cell in each cluster. Shown are the RNA counts (top), unique genes (middle), and mitochondrial RNA fraction (bottom) per cell.

(G) Top7 distinctive genes per cluster. After QC filtering, we calculated the differentially expressed genes (DEGs) in each cluster compared to all other cells. Heatmap shows the expression levels of the top 7 DEGs in up to 500 randomly sampled cells from each cluster.

(H) Shown is the cell fraction by cluster for each vaccine condition (left) and subject (right) using scRNA-seq data.

Data S5 - ScATAC-seq and scRNA-seq analysis of immune response to H5N1/H5N1+AS03, related to Figure 5

